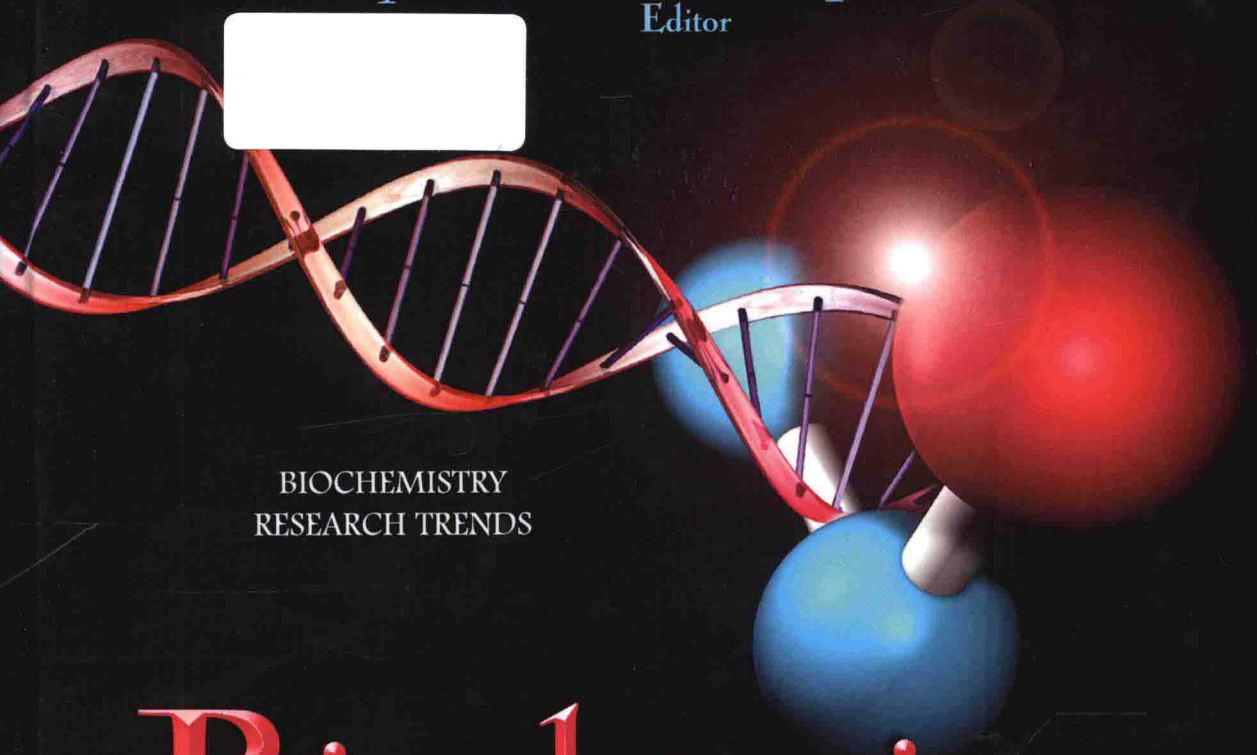
Mary Miu Yee Waye, Ph.D.
Editor



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The Complexity of Human Traits and Diseases

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BIOCHEMISTRY AND MOLECULAR BIOLOGY

THE COMPLEXITY OF HUMAN TRAITS AND DISEASES

MARY MIU YEE WAYE, PHD
EDITOR

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BIOCHEMISTRY RESEARCH TRENDS

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INTRODUCTION

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Even though the ApoA5 gene was discovered more than 13 years ago by Pennacchio et al, 2001 [1], and it was known then the gene influences triglycerides, leading to speculations that it would affect the risk of stroke; the factors that affect cardiovascular diseases are very complex. Larry Baum's group shown that APOA5 genotypes were not significantly associated with strokes or stroke subtypes in the cohort they studied [2]. The discovery of the ApoA5 gene was very special in that, unlike many other genes discovered through studying a known protein or enzyme, advanced bioinformatics was used for the initial gene discovery, an amazing feat that could not have been done without knowledge of the human genome. The laborious effort in deciphering the genome was very controversial in its infancy, and voices of opposition nearly led to premature abortion of the project, and much of the objection was voiced due to the doubt of spending a hugh budget in sequencing what was thought to be useless, i.e., for a major proportion (98%) of "junk DNA" at that time. However, recently it was shown that deleting a non-coding region of what was thought to be "junk DNA" could lead to narrowing of arteries in mice [3] so that might offer an explanation that there are more to the story of stroke risk factors than merely studying APOA5, and such findings just remind us time and again, often the basic research could laid the foundation that is very essential for discoveries made by clinicians or the translational/applied scientists.

This issue described expression of a mammalian dyslexia candidate gene KIAA0319L in insect cells [4], and such expression could help in the study of the mechanism of learning disability. The expression level could be improved in the future and that might need further refinement using knowledge derived from study of the codon bias and its application on the expression of foreign genes in other organisms such as fungi- yeasts, molds and mushrooms [reviewed in 5].

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In another article of this series illustrated the complexity of studying Parkinson's disease (PD) as well [6]. It was found that the BDNF polymorphism might not affect the risk of developing Parkinson's disease as shown previously in other populations. Inconsistencies with different findings of the role of BDNF in different research groups could be due to the fact that physical training could lead to a neuroprotection effect on PD patients and such environmental factors plays an important role in affecting levels of BDNF, pro-BDNF as well as the receptor TrkB in the brains of these PD subjects [7]. Such difficulties in evaluation of confounding factors needs to be considered before we could attempt to design therapeutics that need to introduced cross the blood brain barrier [8].

A review was included in this issue to discuss the relationship between genetic factors and epigenetic factors related to bipolar disorder [9], also known as manic depression. It is clear that many genes are involved in bipolar disorder and that environment factors could play a role and they interact with the genome *via* epigenetic changes in a complex manner. At the end, it is hoped that we will understand the regulatory regions of the genome that could be triggered by environmental factors. More knowledge could be gained if we could subject our hypothetical candidate genes to innovative tests to confirm their role in the disease or complex traits, in much the same manner as how the Nobel laureates of physiology prize awarded in 2014:- Dr. John M. O'Keefe, Dr. May-Britt Moser and Dr. Edvard I. Moser performed optogenetic experiments and succeed in their discoveries of the navigation function of nerve cells in the brain [10].

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CONTENTS

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Chapter 1

APOLIPOPROTEIN A5 POLYMORPHISM AND TRIGLYCERIDES IN ISCHEMIC CEREBROVASCULAR DISEASE

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ABSTRACT

Serum lipids are correlated with certain types of cerebrovascular atherosclerosis. The -1131T>C polymorphism in apolipoprotein A5 (APOA5) is associated with triglyceride levels. We investigated associations among this polymorphism, triglycerides, and stroke in 160 cases of acute ischemic stroke or transient ischemic attack and 157 healthy control subjects. Both patients and controls were Chinese. The APOA5 polymorphism was significantly associated with triglyceride levels both in patients and controls. The average triglyceride level was 70% greater in patients and 92% greater in controls with CC than with TT genotypes. Logistic regression indicated that triglyceride level was an independent risk factor for ischemic stroke in this population. However, APOA5

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genotypes were not significantly associated with strokes or stroke subtypes, though larger studies would be needed to exclude the possibility of a small effect on stroke risk.

ABBREVIATIONS

APOA5 = apolipoprotein A5;
APOA1/C3/A4 = apolipoprotein A1 / apolipoprotein C3 / apolipoprotein A4;
ARIC = atherosclerosis risk in communities;
CI = confidence interval;
CT = computed tomography;
DBP = diastolic blood pressure;
DNA = deoxyribonucleic acid;
dNTP = deoxynucleotide triphosphate;
HDL-C = high-density lipoprotein cholesterol;
LDL-C = low-density lipoprotein cholesterol;
MRA = magnetic resonance angiography;
MRI = magnetic resonance imaging;
OR = odds ratio;
PCR = polymerase chain reaction;
SBP = systolic blood pressure;
SD = standard deviation;
SNP = single nucleotide polymorphism;
TC = total cholesterol;
TCD = transcranial Doppler;
TG = triglyceride;
TIA = transient ischemic attack;
VLDL = very low-density lipoprotein

INTRODUCTION

Stroke is one of the most common causes of mortality and morbidity in both developed and developing countries [1]. Although classical risk factors for stroke, such as hypertension and diabetes mellitus, have been established, there is accumulating evidence that genetic elements also contribute to the pathogenesis of cerebrovascular disease [2]. Genes with an established or potential role in atherosclerotic vascular changes appear to be probable candidates, since atherosclerosis has been proposed to be the major underlying condition responsible for acute occlusive cerebrovascular events [3]. Epidemiological studies have suggested that hypertriglyceridemia is an independent risk factor for atherosclerosis [4]. Some but not all studies have reported a positive association between triglyceride levels and ischemic stroke [5;6]. Thus, genetic factors affecting triglyceride levels are possible risk factors for ischemic stroke. For example, the lipoprotein lipase Ser447Ter polymorphism, which is associated with triglyceride level, has been found to affect risk of atherothrombotic cerebral infarction [7].

Pennacchio et al. [8] identified APOA5, an apolipoprotein gene near the APOA1/C3/A4 gene cluster on human chromosome 11q23. Deletion of the gene in knockout mice raised triglyceride levels by a factor of four, while overexpression of APOA5 in transgenic mice lowered triglyceride levels to one-third of normal [8]. Three single nucleotide polymorphisms (SNPs), located 5' to the gene (-1131T>C), in intron 3 (IVS3+476G>A), and in the 3' untranslated region (1259T>C), were associated with triglyceride levels in humans [8]. The -1131T>C polymorphism of APOA5 has been associated with serum triglycerides in populations of different ethnicities [9;10]. The frequency of the rare allele of APOA5 is higher in Chinese than in Hispanic, European or Caucasian populations [8;9;11;12]. The present study was carried out in order to explore the association of this SNP with serum triglyceride levels and the relationship of APOA5 genotype and triglycerides with ischemic stroke.

METHODS

Patients

One hundred and sixty consecutive Chinese patients with acute ischemic stroke or transient ischemic attack (TIA) from January to June 2002 were recruited following admission to the acute stroke unit of a general regional hospital, Prince of Wales Hospital, Shatin, Hong Kong. Research with humans was carried out according to the principles of the Declaration of Helsinki; informed consent was obtained, and the hospital's institutional review board approved the study. All patients in our study were hospitalized because of acute cerebral infarction within seven days of symptom onset. Brain computed tomography (CT) was done on all patients within 24 hours of admission. Patients with past history or CT feature of intracerebral hemorrhage were excluded. Magnetic resonance imaging, including T1, T2-weighted and magnetic resonance angiography (MRA) was performed for most of the patients (90%), except those who were contraindicated for MRI because of pace maker *in-situ*, refusal, claustrophobia, or unstable medical condition. Ultrasound carotid duplex, extracranial and transcranial Doppler (TCD) ultrasound, and electrocardiography were performed routinely for cerebral infarction patients at this hospital.

All patients underwent a detailed clinical analysis. Age, gender and vascular risk factors, including hypertension, diabetes mellitus, smoking habit and history of hyperlipidaemia, were collected during acute admission for all the patients. Subjects were defined as hypertensive if their systolic blood pressure (SBP) was $\geq$140 mm Hg or diastolic blood pressure (DBP) $\geq$90 mm Hg on at least two occasions, or if they were receiving blood pressure-lowering medication. Patients were considered diabetic if the fasting plasma glucose was $\geq$7.8 mmol/L [13].

Lipid profile including triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), and total cholesterol (TC) were measured during hospitalization, most within 3 days after admission. Low-density lipoprotein cholesterol (LDL-C) level was calculated. Levels of TC (enzymatic method), TG (enzymatic method without glycerol blanking), and HDL-C (dextran sulfate-magnesium chloride precipitation) were measured on a Hitachi analyzer.

Etiology

Following evaluation of the clinical and radiological features, we classified the potential vascular etiologies into the following categories:

1. Large artery disease (intracranial large artery disease or extracranial carotid artery disease). Presence of intracranial large artery disease was determined via MRA or TCD. Intracranial large artery disease was considered if the stenosis was at least moderate (≥ 50%) in severity and if the relevant infarct was located within its arterial supply. Signal void was considered as a severe lesion. The criteria of intracranial stenosis by TCD were given elsewhere [14]. Presence of extracranial carotid artery stenosis was screened by duplex ultrasound. Carotid artery stenosis was considered if the stenosis was ≥50% and the relevant infarct was located within its arterial distribution.
2. Small vessel disease. Patients who had no relevant intracranial large artery disease, extracranial carotid artery stenosis, cardiac embolic sources or other miscellaneous causes (such as inflammatory disorders, hematological disorders or recreational drug abuse), and with small infarct (3-15 mm in major diameter present on CT or MRI) located in a subcortical or brain stem area were presumed to have small vessel disease.
3. Cardioembolism was assigned as the etiology for patients who had concurrent presence of arterial fibrillation, mitral or aortic metallic heart valves, acute congestive heart failure, recent (≤6 weeks before stroke) myocardial infarction, sick sinus syndrome, atrial myxoma, or patent foramen ovale. Electrocardiograms were performed on all patients.
4. Other causes were assigned for patients with double or triple pathology or with incomplete or inadequate examinations to draw any conclusion.

Controls

Among people free of symptomatic cerebrovascular diseases who were selected randomly from community centers for the elderly in Shatin, 157 Chinese subjects matched for age and sex volunteered to participate as controls. Venous blood samples from patients and controls were taken in the morning after a 12h fast. All subjects gave written, informed consent to participate in the study, which was approved by the ethics review committee of the hospital.

Genotyping

DNA was extracted from blood. Genotyping was performed as described previously (as SNP3) [8;9]: 200 μM dNTP, 1 unit TaqGold (Applera), 1X TaqGold Buffer, 400 nM AV6F primer (GATTGATTCAAGATGCATTTAGGAC), 400 nM AV6R primer (CCCCAGGAACTGGAGCGAAATT), 1.5 mM $MgCl_2$, and 1 μl DNA were mixed and the

volume adjusted to 20 μl with water. PCR was performed at 95°C for 12 min followed by 30 cycles of 94°C for 15 sec, 55°C for 30 sec, and 72°C for 30 sec, followed by 72°C for 3 min. Products were digested with TruII or MseI and resolved on 3% agarose gels post-stained with SYBR Gold (Molecular Probes).

Statistics

SPSS version 10.0 and Epi6 (World Health Organization, Geneva, Switzerland) were used for data analysis. Differences in anthropometric and fasting plasma biochemical parameters between patients and controls and among the genotypes were examined using Student's t test and analysis of variance.

Chi-squared analysis was used to compare the genotype distribution between patients and controls and among ischemic stroke subtypes in patients. Comparison of triglyceride levels between patients and controls was performed by Mann-Whitney U test. Kruskal Wallis test was used for comparisons of triglyceride concentration among APOA5 genotypes. To determine the variables correlated with ischemic stroke, we applied multivariate logistic regression. We included all variables that were significant in univariate analysis. These variables included hypertension, diabetes and triglyceride level. Age and gender were also included in the analyses. Because the distribution of triglyceride level was skewed, the natural logarithm of the triglyceride level was introduced into the model.

RESULTS

The study population characteristics are described in Table 1. Stroke patients and controls were matched for age and sex. Both systolic and diastolic blood pressures, as well as frequencies of hypertension and diabetes mellitus, were significantly higher in patients. Triglyceride levels were higher in patients than controls ($p<0.001$).

Table 1. Characteristics of patients and control subjects

	Patients (n=160)	Control (n=157)	p
Age (SD)	70.3 (11.5)	70.0 (2.9)	0.47
Male (%)	83 (51.9)	75 (47.8)	0.46
Hypertension (%)	107 (66.9)	74 (47.1)	<0.001
Diabetes mellitus (%)	49 (30.6)	20 (13.2)	<0.001
Lipid concentration, mmol/L			
TG (SD)	1.75 (0.99)	1.45 (0.95)	<0.001
TC (SD)	5.62 (1.14)	5.42 (0.93)	0.100
HDL-C (SD)	1.35 (0.35)	1.31 (0.36)	0.29
LDL-C (SD)	3.52 (0.99)	3.48 (0.82)	0.72

TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TG, triglyceride

Significant differences in triglyceride levels were found among TT, TC and CC genotypes, and the CC genotype was associated with higher triglyceride levels both in patients and in healthy controls (Table 2). The average triglyceride level was 70% greater in patients and 92% greater in controls with CC than with TT genotypes. When patients with allele C are pooled and compared with non-C carriers, allele C carriers are associated with higher triglyceride concentration in controls ($p<0.001$), but not in patients ($p=0.29$).

Table 2. Association of APOA5 genotypes with lipid levels in patients and control subjects

	TT	TC	CC	p
Patients				
TG, mmol/L	1.66 (0.93)	1.69 (0.88)	2.83 (1.60)	0.013
TC, mmol/L	5.63 (1.13)	5.61 (1.08)	5.56 (1.71)	0.98
HDL, mmol/L	1.39 (0.34)	1.32 (0.36)	1.30 (0.35)	0.54
LDL, mmol/L	3.50 (1.00)	3.60 (0.90)	2.97 (1.48)	0.28
Controls				
TG, mmol/L	1.27 (0.96)	1.52 (0.73)	2.44 (0.95)	<0.001
TC, mmol/L	5.52 (0.98)	5.21 (0.87)	5.84 (0.49)	0.031
HDL, mmol/L	1.43 (0.37)	1.19 (0.29)	1.13 (0.39)	<0.001
LDL, mmol/L	3.57 (0.86)	3.32 (0.79)	3.79 (0.56)	0.10

P values for analysis of variance among APOA5 genotypes. Standard deviations in parentheses

This lack of significance may be explained by the similar triglyceride levels in patients with TT and TC genotypes. No significant differences in cholesterol, HDL cholesterol or LDL cholesterol were found among APOA5 genotypes in patients. However, in controls, the total cholesterol ($p=0.031$) and HDL cholesterol levels ($p<0.001$) were different among the genotypes. Comparing controls with CC genotypes with controls having the TT genotype, the average total cholesterol level was 6% higher, and the average HDL level was 21% lower.

Table 3. Frequency of APOA5 genotype in patients and control subjects

	n	TT	TC	CC	P
Control	157	81 (51.6)	66 (42.2)	10 (6.4)	
All patients	160	84 (52.5)	67 (41.9)	9 (5.6)	0.96
Stroke subtype					
Large-vessel disease	58	30 (51.7)	24 (41.4)	4 (6.9)	0.99
Small-vessel disease	49	30 (61.2)	17 (34.7)	2 (4.1)	0.48
Cardioembolism	12	5 (41.7)	6 (50)	1 (8.3)	0.80
Other cause	41	19 (46.3)	20 (48.8)	2 (4.9)	0.73

Chi-square test for comparing genotype distribution between controls and patients as a whole or in subgroups. Percentages in parentheses

Table 3 shows the distribution of APOA5 genotypes according to ischemic stroke subgroups. No significant differences were found between cases and controls in the frequencies of APOA5 genotypes (χ^2=0.086, df=2, p=0.96) or alleles (χ^2=0.46, df=1, p=0.49).

The odds ratio for the association of the C allele with stroke was 0.96, with a 95% confidence interval of 0.66 to 1.38. There were no significant differences in allele frequencies between the sexes in controls (χ^2=0.64, df=2, p=0.73) or in patients (χ^2=0.21, df=2, p=0.90). In patients with large-vessel and cardioembolism etiology, there were trends toward higher frequencies of TC and CC genotypes compared with those with small-vessel disease, but the differences were not significant. No significant differences were observed among ischemic subgroups in triglyceride level (χ^2=0.45, df=3, p=0.72).

Conditional forward logistic regression showed that after adjusting for age and gender, the following variables were associated with ischemic stroke: history of hypertension (OR=1.93, 95% CI 1.19-3.13), diabetes mellitus (OR=2.47, 95% CI 1.36-4.50) and triglyceride level. An increase of 1 natural log unit (2.72x) in triglyceride level was associated with an adjusted OR of 2.22 (95% CI 1.35-3.63).

CONCLUSION

Previous studies showed that APOA5 -1131T>C is significantly associated with plasma triglyceride levels in healthy subjects [9;15], school children [16], and individuals with hypertriglyceridemia [17] or familial combined hyperlipidemia [10]. The purpose of our previous study of this polymorphism was to examine its association with triglyceride levels in healthy subjects [9], while our current study aims to examine its association with the risk of stroke. Our results indicated that the CC genotype was associated with higher triglyceride levels both in stroke patients and control subjects. The association between APOA5 genotype and HDL cholesterol or LDL cholesterol was inconsistent. A number of studies have demonstrated that the rare allele (C) of APOA5 was associated with specific lipoprotein TG and VLDL cholesterol [11] and inversely associated with HDL cholesterol [11;12], while another study [15] failed to find significant difference either in the serum total cholesterol or the LDL and HDL cholesterol among APOA5 genotypes. In the present study, we found the minor allele of APOA5 was inversely associated with HDL cholesterol in controls, which is comparable to previous studies [11;12]. However, no significant association between APOA5 genotype and lipid profiles except for triglyceride was found in stroke patients. This may be due to the relatively small number of patients and lack of statistical power to detect a small effect. Alternatively, it could be due to disease confounders or due to the stroke and subsequent treatment, such as lipid-lowering therapy, affecting the lipid profiles. Larger studies would provide more power to detect small effects, while prospective studies that measure lipid profiles before the stroke may help to clarify the latter possible explanation.

The results suggested that APOA5 variation may be an important factor in determining lipid levels in Chinese. The mechanism by which this polymorphism could affect serum triglyceride and HDL cholesterol levels and propensity to atherosclerosis remains poorly understood. Perhaps –1131C, or a polymorphism in linkage disequilibrium with –1131C [18],